Supporting Information

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Boosting the Throughput of Separation Techniques by Multiplexing

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Content

1	General Instrumentation	2
2	Six-port Continuous Flow Multiplexing Injector	2
2.1	Design.....	2
2.2	Performance Tests	3
3	Multiplexing Experiments	5
3.1	Generation of the Multiplexing Sequence	5
3.2	Generation of the High-Throughput Multiplexing Sequence	5
3.3	Control of the Multiplexing Injector	6
4	Data Acquisition.....	7
5	Data Deconvolution.....	7
5.1	Overview of the Data Deconvolution Algorithm	7
5.2	Hadamard Transformation.....	9
5.3	Chromatographic Data	10
5.4	The Matrix/ Linear Equation-System	10
5.5	Solving the Matrix/ Linear Equation-System: Gauss-Jordan-Algorithm	12
5.6	Statistical Analysis of Errors and Deviations	14
5.7	Results	14
5.8	Sample Complexity and Deconvolution.....	17
6	References	18

1 General Instrumentation

All GC measurements were performed on a TRACE GC (Thermo, San Jose, California, USA) equipped with a split injector and a flame ionization detector (both at 250°C). The GC settings were controlled by the Xcalibur software (Thermo, San Jose, California, USA).

All separations were performed on an HP-5 fused-silica column (Agilent, Palo Alto, USA) coated with (5%-phenyl)-methylpolysiloxane (30 m × 0.32 mm i.d., 0.25 µm film thickness) at 80°C and 80 kPa head pressure. Helium was used as carrier gas, the split ratio was set to 1:200. Data was acquired at 10 Hz.

2 Six-port Continuous Flow Multiplexing Injector

2.1 Design

The experimental setup consists of a laboratory built six-channel continuous-flow split/ splitless injector with 6 heated sample ports (250°C), separately controlled by fast 3/ 2 way solenoid valves (SMC corporation, Tokyo, Japan). The injector was manufactured from brass and is equipped with 2 heating cartridges and a thermocouple to exactly control temperature. The sample ports are equipped with glass sleeves, treated with chlorotrimethylsilane (Fluka, Buchs, Switzerland) to minimize analyte surface interactions.

This multiplexing injector is directly mounted onto the split/splitless injector of the GC.

Samples were continuously pumped through fused-silica capillaries (0.05 mm i.d.) into the sample ports of the multiplexing injector and there continuously evaporated. The samples are repetitively injected into the split/ splitless injector of the GC by short pressure pulses in the range of 1 to 10 ms with He (400 kPa) according to the bar-codelets of the binary pseudo-random sequence. The multiplexing injector is purged through the injection needle by the split/ splitless injector of the gas chromatograph to prevent sample mixing.

Supplementary Figure 1. Design and performance of the six-channel continuous-flow split/splitless multiplexing injector. (A) Cross-section of the multiplexing injector consisting of heated sample ports controlled by fast solenoid valves. (B) Experimental chromatogram of a sample containing 5 analytes obtained by repetitive injections (time bin interval 10 s) with the multiplexing injector. The dashed lines show the signal stability of the individual analyte peaks.

2.2 Performance Tests

As already shown in the design section several tests were performed concerning the injection stability, the injection interval Δt and sample mixing. The injection stability and reproducibility is shown in **Supplementary Figure 2**, **Supplementary Figure 3**, and **Supplementary Figure 4** for a single analyte and different analytes showing characteristic steps for the individual concentrations of the analytes. The injection interval Δt can be varied between 500 ms and 60 s. Faster modulation is possible for fast separations in the range of only a few seconds.

Supplementary Figure 2. Injection stability of a single sample (conventional multiplexing chromatogram) with long injection intervals (7-bit sequence = 127 elements, $\Delta t = 20$ s, 10 ms pulse).

Supplementary Figure 3. Injection stability of 6 different samples with long injection intervals (11-bit sequence = 2047 elements, $\Delta t = 10$ s, 1 ms pulse, 50 repetitive injections per sample).

Supplementary Figure 4. Zoomed in chromatogram (11-bit sequence = 2047 elements, $\Delta t = 10$ s, 1 ms pulse, 50 repetitive injections per sample).

Sample mixing is effectively prevented by the counter current gas stream of the GC split/splitless injector. In these experiments mixing was not observed.

3 Multiplexing Experiments

3.1 Generation of the Multiplexing Sequence

The n -bit binary pseudo-random sequences used throughout all experiments consist of N elements ($N = 2^n - 1$) with $2^{n/2} - 1$ elements 0 and $2^{n/2} - 1$ elements 1, which are derived from an $(n+1) \times (n+1)$ Hadamard matrix.¹ These sequences are constructed by the use of a virtual shift register applying logical operations. A complete description of the algorithms used here is given in reference.² The modulation sequence was generated by a program written in Delphi (Borland, Cupertino, USA). Only for elements equal to 1 sample injections are performed. This multiplexing sequence represents the bar-code, which conserves only the information of the single analytes, but not the concentrations of the individual samples.

3.2 Generation of the High-Throughput Multiplexing Sequence

Supplementary Figure 5. Creating a high-throughput multiplexing sequence from a conventional multiplexing sequence.

To increase the sample throughput the conventional multiplexing sequence (bar-code) is divided into bar-codelets with the same number of repetitive injections. These repetitive injections are necessary for the unambiguous identification of the individual samples. To determine the number of necessary injections per sample, equations were derived and are given in the main manuscript. Here a detailed derivation of these equations is given. The general concept of these equations is that a linear equation system, which results from such experiments, can only be solved if there are more independent data points in a chromatogram available than unknown analyte concentrations. Furthermore these equations also characterize the performance of high-throughput multiplexing gas chromatography (htMPGC). With these equations it can be shown that for fast separations the sample throughput can be inherently increased.

The duration $t_{\max}$ to perform a htMPGC analysis is defined by the sequence length N , the time bin interval Δt and the maximum retention time $t_R^{\max}$ [min] of the analytes according to equation (1).

$$t_{\max} = N \cdot \Delta t + t_R^{\max} \quad (1)$$

For the unambiguous determination of a single analyte j of sample i the product ($j_{\max} \times i_{\max}$) of the maximum numbers of analytes $j_{\max}$ and maximum number of samples $i_{\max}$ must not exceed the maximum number of data points acquired by htMPGC. The number of data points can be calculated from the sum of the sequence length N and the maximum retention time $t_R^{\max}$ divided by the time bin interval Δt , and multiplied by the data acquisition frequency f [Hz]. Therefore, the maximum number of samples $i_{\max}$ that can be unambiguously determined is given by equation (2).

$$i_{\max} = f \frac{N + \frac{t_R^{\max}}{\Delta t}}{j_{\max}} \quad (2)$$

The minimum number of repetitive injections $r_{\min}$ is defined by the number of elements 1 in the modulation sequence and the maximum number of samples $i_{\max}$:

$$r_{\min} = \frac{N}{2i_{\max}} \quad (3)$$

This equation is important to divide the bar-code into bar-codelets of a certain length. After at least $r_{\min}$ sample injections the injection pulses are shifted to the next channel. The number of repetitive injections is saved together with all other chromatographic parameters and is needed for the deconvolution of the raw data.

The throughput power P of a htMPGC analysis is expressed by the ratio of the maximum number of samples $i_{\max}$ and the total duration $t_{\max}$:

$$P = \frac{i_{\max}}{t_{\max}} \quad (4)$$

3.3 Control of the Multiplexing Injector

The modulation sequences were transmitted by a program written in Delphi (Borland, Cupertino, USA) to an USB computer interface Labjack U12 with an extension card (Labjack, Lakewood, USA) allowing to address 20 digital I/O channels. The signal of this AD/DA converter is amplified by a transistor network and directly transmitted to the solenoid valves of the multiplexing injector. These signals are between 1 to 20 ms broad and open the solenoid valves for a pressure pulse to transfer the sample volume into the split/splitless injector of the TRACE GC.

Supplementary Figure 6. Experimental raw chromatogram obtained by htMPGC. 11-bit (2,047 time bins) binary pseudo-random sequence with injection pulses of 1 ms. Data was acquired with a FID at a rate of 10 Hz. Time bin interval $\Delta t = 1$ s (1,800 injections/ h), 10 injections/ sample (150 samples/ h). In this experiment 102 samples from 6 channels were analyzed.

Supplementary Figure 7. Experimental chromatographic data obtained by htMPGC. 200 samples with 5 analytes of varying concentrations were injected by the six-channel continuous-flow split/splitless multiplexing injector according to a 11-bit (2,047 time bins) binary pseudo-random sequence with injection pulses of 1 ms. Data was acquired with a FID at a rate of 10 Hz. Time bin interval $\Delta t = 0.6$ s (3,000 injections/ h), 5 injections/ sample (453 samples/ h).

4 Data Acquisition

Data of the flame ionization detector (FID) was acquired at 10 Hz with the Xcalibur software (Thermo, San Jose, California, USA). The data acquisition was started by the software controlling the multiplexing injector. The data trace of the FID was converted into ASCII format by a tool programmed by Thermo. The data in ASCII format were used for the data deconvolution, described in the next chapter.

5 Data Deconvolution

5.1 Overview of the Data Deconvolution Algorithm

The flow chart of the data deconvolution process is given in **Supplementary Figure 8**. The HT of the raw chromatogram gives an overview chromatogram with the peaks of the analytes. The peak information of the analytes (retention times and peak shapes) is used to fill matrix $\Phi_{A_{i,j}}(t)$ with normalized peak profiles according to the structured modulation sequence. Each row represents the chromatogram of the repetitive injection of a single analyte of a single sample. Multiplication of this matrix with the analytes concentrations vector of all samples equals the raw chromatogram represented as vector $I(t)$. This linear equation system is solved by a Gauss-Jordan algorithm to obtain the analytes

concentrations to calculate conventional chromatograms (top, right, chromatogram of sample 7) of the individual samples.

Supplementary Figure 8. Flow-chart of the data deconvolution process of chromatograms obtained by htMPGC to determine the relative analyte concentrations of the individual samples.

Supplementary Figure 9 summarizes the steps of the data deconvolution algorithm.

Supplementary Figure 9. Flow-chart of the deconvolution algorithm.

This algorithm consists of several steps. In the first step the raw data are converted into ASCII format and the data are normalized to 100 for further data processing. In the next step a Hadamard transformation is performed to get an overview chromatogram providing data about number of analytes in unknown mixtures, retention times of the analytes and their peak width to calculate separation efficiencies necessary to calculate in the next step the rows of the matrix system. Each row contains the repetitive injection of a single analyte according to the individual bar-codelet (time shifting of these packages). Multiplication of this matrix with the concentrations of the analytes to be determined gives the raw chromatogram. For the data deconvolution it is necessary to divide this matrix in sub-matrices which can be solved by a Gauss-Jordan algorithm. As a result the concentration vector is obtained.

5.2 Hadamard Transformation

After the separation, the conventional chromatogram is obtained by Hadamard transformation of the $2^{n/2}$ overlapping chromatograms according to equation (5). Therefore the raw chromatogram is transformed into the circular representation which means that the time bins greater than the sequence length multiplied by the oversampling factor (data acquisition rate 10 Hz) are added to the beginning of the chromatogram. This chromatogram is then multiplied by the inverse simplex matrix obtained from the corresponding Hadamard matrix. Detailed information about the Hadamard transformation is given in reference 2.

$$\begin{bmatrix} \text{Overview} \\ \text{Chromatogram} \end{bmatrix} = \begin{bmatrix} S \end{bmatrix}_n^{-1} \times \begin{bmatrix} \text{Raw} \\ \text{Chromatogram} \end{bmatrix} \quad (5)$$

The Hadamard transformation is performed by a program written in Delphi.

Supplementary Figure 10. Hadamard transformed chromatogram. 200 samples with 5 analytes of varying concentrations were injected by the six-channel continuous-flow split/splitless multiplexing injector according to a 11-bit (2,047 time bins) binary pseudo-random sequence with injection pulses of 1 ms. Data was acquired with a FID at a rate of 10 Hz. Time bin interval $\Delta t = 0.6$ s (3,000 injections/ h), 5 injections/ sample (453 samples/ h).

5.3 Chromatographic Data

From the Hadamard transformed overview chromatogram the chromatographic peak data are obtained by a standard integration procedure determining the apex of the peaks (retention times), peak width and analyzing their peak shape. These data are used to calculate single peak profiles of the individual peaks.

Supplementary Figure 11. Smoothed zoom in of a Hadamard transformed chromatogram. 200 samples with 5 analytes of varying concentrations were injected by the six-channel continuous-flow split/splitless multiplexing injector according to a 11-bit (2,047 time bins) binary pseudo-random sequence with injection pulses of 1 ms. Data was acquired with a flame ionization detector (FID) at a rate of 10 Hz. Time bin interval $\Delta t = 0.6$ s (3,000 injections/ h), 5 injections/ sample (453 samples/ h).

Supplementary Figure 12. Analysis of chromatographic data.

5.4 The Matrix/ Linear Equation-System

To deconvolute the raw data in order to obtain the individual concentrations of the single analytes in the individual samples it is necessary to solve a linear equation system using the chromatographic data obtained by the Hadamard transformation.

Each row of this matrix represents the repetitively injected elution profiles of an analyte i in sample j . Each column represents the time bin (elution time). The matrix elements represent the signal intensity

which is normalized to 100 for the deconvolution process. Multiplication of this matrix with the unknown concentration vector, which holds the information of the concentration of the individual analytes i in the samples j gives the raw chromatogram.

The peak shape, the retention time t_R^j , peak width at half height w_h^j from the overview chromatograms are used as input parameters for the data processing to obtain time-resolved analyte peak areas of the individual samples (Fig. 4). The raw chromatogram is a convolution of the elution profiles $\varphi_{A_{i,j}}(t)$ of the time shifted analytes $A_{i,j}$ (analyte j in sample i). For the mathematical separation of the different samples and their analytes a matrix $\Phi_{A_{i,j}}(t)$ of the dimension $(t_{max}/\Delta t) \times (i_{max} \times j_{max})$ is created, where the rows are the sum of the time shifted elution profiles $\varphi_{A_{i,j}}(t)$ of the repetitively injected analyte j of sample i according to the bar-codelet of the structured modulation sequence (Equation 6):

$$\Phi_{A_{i,j}}(t) = \sum_{i=1}^{i_{max}} \sum_{j=1}^{j_{max}} \varphi_{A_{i,j}}(t) \quad (6)$$

The matrix $\Phi_{A_{i,j}}(t)$ represents a linear equation system, which describes the signal intensity of the individual analytes at a certain retention time.

The multiplication of this normalized analyte peak shape matrix $\Phi_{A_{i,j}}(t)$ with the analyte concentrations vector A gives the time-dependent intensities $I(t)$ of the raw chromatogram (Equation 7 and Equation 8).

$$\Phi_{A_{i,j}}(t) \times A = I(t) \quad (7)$$

The deconvolution algorithm consists of the following steps: (i) calculation of the matrix size from the number of peaks and samples, (ii) creation of the matrix, (iii) filling of the matrix rows by calculation of the individual analytes peaks according to the peak profiles, retention times and time shift of the samples and (iv) creation of the concentration vector with statistical information about deviations.

$$\begin{aligned} Chromatogramm &= ij \sum_{k=1}^{k_{max}} \Phi(A_k) \\ &= \begin{bmatrix} \varphi(A_1(1)) & \varphi(A_2(1)) & \varphi(A_3(1)) & \cdot & \cdot & \cdot & \cdot & \varphi(A_k(1)) \\ \varphi(A_1(2)) & \varphi(A_2(2)) & \varphi(A_3(2)) & & & & & \cdot \\ \varphi(A_1(3)) & \varphi(A_2(3)) & \varphi(A_3(3)) & & & & & \cdot \\ \cdot & & & \cdot & & & & \cdot \\ \cdot & & & & & & & \cdot \\ \cdot & & & & \varphi(A_{k-2}(t-2)) & \varphi(A_{k-1}(t-2)) & \varphi(A_k(t-2)) & A_{i,1,2} \\ \cdot & & & & \varphi(A_{k-2}(t-1)) & \varphi(A_{k-1}(t-1)) & \varphi(A_k(t-1)) & A_{i,j,2} \\ \varphi(A_1(t)) & \cdot & \cdot & \cdot & \cdot & \varphi(A_{k-2}(t)) & \varphi(A_{k-1}(t)) & \varphi(A_k(t)) \end{bmatrix} \begin{bmatrix} A_{1,1,1} \\ A_{i,1,1} \\ A_{1,2,1} \\ A_{i,j,1} \\ A_{1,1,2} \\ A_{i,1,2} \\ A_{i,j,2} \\ A_{i,j,k} \end{bmatrix} = \begin{bmatrix} I_1 \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ I_t \end{bmatrix} \quad (8) \end{aligned}$$

5.5 Solving the Matrix/ Linear Equation-System: Gauss-Jordan-Algorithm

The matrix and the resulting linear equation system is solved by application of a Gauss-Jordan algorithm, described in detail in reference 3. Because of the size of the here created matrices I found it more convenient to separate the matrix in sub matrices that can be solved by a Gauss-Jordan algorithm of 2nd, 3rd, 4th, or 5th order. The program written in Delphi can handle Gauss-Jordan algorithms of any order, but for the optimization of computational time smaller matrices are favored. Therefore the algorithm shifts from time bin to time bin analyzing if a matrix element is filled or not and how many unknown concentrations and equations are available. If the number of equations is bigger than the number of unknown parameters the algorithm reduces the number of equations by summing two equations. **Supplementary Figure 13** and **Supplementary Figure 15** show this step-by-step process graphically for an example.

Supplementary Figure 13. Selected sections of the chromatogram during the deconvolution process. 1st order Gauss-Jordan algorithm. Left: The section is depicted before solving the partial linear equation system of this time region. Right: Section of the chromatogram with solved linear equation system.

Supplementary Figure 14. Selected sections of the chromatogram during the deconvolution process. 2nd order Gauss-Jordan algorithm. Left: The section is depicted before solving the partial linear equation system of this time region. Right: Section of the chromatogram with solved linear equation system.

The solution of the sub matrices is the used to adjust the determined concentrations of the analytes matrix $\Phi_{A_i,j}(t)$ until all concentrations are determined.

Supplementary Figure 15. Completely evaluated chromatogram (green: calculated data, red: deviation, black (almost hidden): raw data).

Supplementary Figure 15 and *Supplementary Figure 16* shows a completely evaluated chromatogram after application of the here described algorithm.

Supplementary Figure 16. Zoom in of completely evaluated chromatogram (green: calculated data, red: deviation, black (almost hidden): raw data).

5.6 Statistical Analysis of Errors and Deviations

The deviation is calculated by subtracting the experimental raw chromatogram from the evaluated data and by subtracting the peak areas of the individual peaks. In *Supplementary Figure 15* the deviation is visualized as a red curve.

5.7 Results

Supplementary Figure 17 shows a htMPGC experiment with 6 different samples, repetitively injected to compare injection stability and repeatability of the sample injections with two analytes. In **Table 1** the results of this analysis is summarized.

Supplementary Figure 17. left: Experimental raw chromatogram obtained by htMPGC. 11-bit (2,047 time bins) binary pseudo-random sequence with injection pulses of 1 ms. Data was acquired with a FID at a rate of 10 Hz. Time bin interval $\Delta t = 1$ s (1,800 injections/ h), 10 injections/ sample (150 samples/ h). 6 samples from 6 channels were repetitively analyzed for comparison. Right: deconvoluted chromatogram.

Table 1. Summarized results.

Sample	Analyte 1	Analyte 2	Analyte 1	Analyte 2
Injection	[counts]	[counts]	[%]	[%]
1	$2.69 \cdot 10^7$	$5.51 \cdot 10^7$	32.80	67.20
2	$1.98 \cdot 10^7$	$8.00 \cdot 10^7$	19.82	80.18
3	$1.46 \cdot 10^7$	$7.30 \cdot 10^7$	16.65	83.35
4	$7.97 \cdot 10^6$	$1.72 \cdot 10^7$	31.71	68.29
5	$3.43 \cdot 10^7$	$4.19 \cdot 10^7$	45.00	55.00
6	$4.13 \cdot 10^7$	$3.02 \cdot 10^7$	57.71	42.29
7	$2.70 \cdot 10^7$	$5.50 \cdot 10^7$	32.93	67.07
8	$1.97 \cdot 10^7$	$8.02 \cdot 10^7$	19.72	80.28
9	$1.43 \cdot 10^7$	$7.32 \cdot 10^7$	16.31	83.69
10	$7.84 \cdot 10^6$	$1.73 \cdot 10^7$	31.18	68.82
11	$3.42 \cdot 10^7$	$4.19 \cdot 10^7$	44.95	55.05
12	$4.13 \cdot 10^7$	$3.04 \cdot 10^7$	57.56	42.44
13	$2.68 \cdot 10^7$	$5.51 \cdot 10^7$	32.74	67.26
14	$1.97 \cdot 10^7$	$8.02 \cdot 10^7$	19.69	80.31
15	$1.43 \cdot 10^7$	$7.34 \cdot 10^7$	16.29	83.71
16	$7.68 \cdot 10^6$	$1.73 \cdot 10^7$	31.75	68.25
17	$3.43 \cdot 10^7$	$4.18 \cdot 10^7$	45.07	54.93
18	$4.14 \cdot 10^7$	$3.01 \cdot 10^7$	57.92	42.08
19	$2.71 \cdot 10^7$	$5.49 \cdot 10^7$	33.07	66.93
20	$1.98 \cdot 10^7$	$8.01 \cdot 10^7$	19.81	80.19
21	$1.45 \cdot 10^7$	$7.30 \cdot 10^7$	16.59	83.41
22	$8.16 \cdot 10^6$	$1.71 \cdot 10^7$	31.25	68.75
23	$3.43 \cdot 10^7$	$4.20 \cdot 10^7$	44.93	55.07
24	$4.11 \cdot 10^7$	$3.04 \cdot 10^7$	57.47	42.53
25	$2.68 \cdot 10^7$	$5.49 \cdot 10^7$	32.80	67.20
26	$1.97 \cdot 10^7$	$8.02 \cdot 10^7$	19.74	80.26

27	$1.44 \cdot 10^7$	$7.33 \cdot 10^7$	16.44	83.56
28	$7.58 \cdot 10^6$	$1.75 \cdot 10^7$	31.19	68.81
29	$3.41 \cdot 10^7$	$3.77 \cdot 10^7$	45.52	54.48
30	$5.53 \cdot 10^7$	$1.47 \cdot 10^7$	78.98	21.02
31	$3.74 \cdot 10^7$	$5.14 \cdot 10^7$	32.11	67.89
32	$1.97 \cdot 10^7$	$8.45 \cdot 10^7$	18.94	81.06
33	$1.19 \cdot 10^7$	$7.41 \cdot 10^7$	16.89	83.11
34	$6.98 \cdot 10^6$	$1.88 \cdot 10^7$	31.10	68.90
35	$3.18 \cdot 10^7$	$4.55 \cdot 10^7$	45.10	54.90
36	$3.62 \cdot 10^7$	$3.18 \cdot 10^7$	57.23	42.77
37	$2.98 \cdot 10^7$	$4.75 \cdot 10^7$	32.52	67.48
38	$2.48 \cdot 10^7$	$7.51 \cdot 10^7$	19.85	80.15
39	$2.54 \cdot 10^7$	$5.81 \cdot 10^7$	16.40	83.60
40	$2.10 \cdot 10^7$	$1.10 \cdot 10^7$	31.62	68.38
41	$3.59 \cdot 10^7$	$4.18 \cdot 10^7$	45.17	54.83
42	$4.02 \cdot 10^7$	$3.18 \cdot 10^7$	57.84	42.16
43	$2.57 \cdot 10^7$	$5.58 \cdot 10^7$	32.54	67.46
44	$1.90 \cdot 10^7$	$8.10 \cdot 10^7$	19.01	80.99
45	$1.36 \cdot 10^7$	$7.35 \cdot 10^7$	16.61	83.39
46	$7.59 \cdot 10^6$	$1.76 \cdot 10^7$	31.08	68.92
47	$3.39 \cdot 10^7$	$4.21 \cdot 10^7$	45.61	54.39
48	$4.09 \cdot 10^7$	$3.05 \cdot 10^7$	57.29	42.71
49	$2.68 \cdot 10^7$	$5.53 \cdot 10^7$	32.63	67.37
50	$1.93 \cdot 10^7$	$8.06 \cdot 10^7$	19.34	80.66
51	$1.42 \cdot 10^7$	$7.31 \cdot 10^7$	16.30	83.70
52	$7.91 \cdot 10^6$	$1.73 \cdot 10^7$	31.41	68.59
53	$3.41 \cdot 10^7$	$4.20 \cdot 10^7$	45.81	54.19
54	$4.13 \cdot 10^7$	$3.00 \cdot 10^7$	57.92	42.08
55	$2.72 \cdot 10^7$	$5.47 \cdot 10^7$	33.19	66.81
56	$2.04 \cdot 10^7$	$7.97 \cdot 10^7$	19.38	80.62
57	$1.48 \cdot 10^7$	$7.28 \cdot 10^7$	16.93	83.07
58	$8.55 \cdot 10^6$	$1.66 \cdot 10^7$	31.99	68.01
59	$3.50 \cdot 10^7$	$4.14 \cdot 10^7$	45.85	54.15
60	$4.16 \cdot 10^7$	$3.00 \cdot 10^7$	58.08	41.92
61	$2.71 \cdot 10^7$	$5.49 \cdot 10^7$	32.99	67.01
62	$1.98 \cdot 10^7$	$8.03 \cdot 10^7$	19.75	80.25
63	$1.42 \cdot 10^7$	$7.36 \cdot 10^7$	16.14	83.86
64	$7.53 \cdot 10^6$	$1.84 \cdot 10^7$	31.03	68.97
65	$3.07 \cdot 10^7$	$4.56 \cdot 10^7$	45.23	54.77
66	$3.96 \cdot 10^7$	$3.13 \cdot 10^7$	57.80	42.20
67	$2.51 \cdot 10^7$	$5.73 \cdot 10^7$	32.47	67.53
68	$1.83 \cdot 10^7$	$8.07 \cdot 10^7$	19.52	80.48
69	$1.36 \cdot 10^7$	$7.40 \cdot 10^7$	16.50	83.50
70	$6.68 \cdot 10^6$	$1.80 \cdot 10^7$	31.06	68.94
71	$3.41 \cdot 10^7$	$4.21 \cdot 10^7$	45.73	54.27
72	$4.11 \cdot 10^7$	$3.03 \cdot 10^7$	57.55	42.45
73	$2.71 \cdot 10^7$	$5.47 \cdot 10^7$	33.10	66.90
74	$2.00 \cdot 10^7$	$8.01 \cdot 10^7$	19.99	80.01

75	$1.44 \cdot 10^7$	$7.31 \cdot 10^7$	16.45	83.55
76	$7.97 \cdot 10^6$	$1.91 \cdot 10^7$	31.44	68.56
77	$2.96 \cdot 10^7$	$4.51 \cdot 10^7$	45.61	54.39
78	$4.10 \cdot 10^7$	$3.06 \cdot 10^7$	57.29	42.71
79	$2.67 \cdot 10^7$	$5.53 \cdot 10^7$	32.58	67.42
80	$1.95 \cdot 10^7$	$8.04 \cdot 10^7$	19.51	80.49
81	$1.39 \cdot 10^7$	$7.38 \cdot 10^7$	15.89	84.11
82	$7.49 \cdot 10^6$	$1.75 \cdot 10^7$	31.01	68.99
83	$3.41 \cdot 10^7$	$4.21 \cdot 10^7$	44.77	55.23
84	$4.11 \cdot 10^7$	$3.03 \cdot 10^7$	57.55	42.45
85	$2.70 \cdot 10^7$	$5.50 \cdot 10^7$	32.96	67.04
86	$1.98 \cdot 10^7$	$8.01 \cdot 10^7$	19.81	80.19
87	$1.44 \cdot 10^7$	$7.33 \cdot 10^7$	16.43	83.57
88	$7.85 \cdot 10^6$	$1.73 \cdot 10^7$	31.26	68.74
89	$3.43 \cdot 10^7$	$4.20 \cdot 10^7$	44.91	55.09
90	$4.11 \cdot 10^7$	$3.04 \cdot 10^7$	57.47	42.53
91	$2.69 \cdot 10^7$	$5.49 \cdot 10^7$	32.93	67.07
92	$2.00 \cdot 10^7$	$8.01 \cdot 10^7$	19.95	80.05
93	$1.44 \cdot 10^7$	$7.31 \cdot 10^7$	16.47	83.53
94	$7.96 \cdot 10^6$	$1.71 \cdot 10^7$	31.76	68.24
95	$3.44 \cdot 10^7$	$4.16 \cdot 10^7$	45.21	54.79
96	$4.14 \cdot 10^7$	$3.02 \cdot 10^7$	57.80	42.20
97	$2.70 \cdot 10^7$	$5.50 \cdot 10^7$	32.90	67.10
98	$2.00 \cdot 10^7$	$8.04 \cdot 10^7$	19.92	80.08
99	$1.28 \cdot 10^7$	$7.63 \cdot 10^7$	16.37	83.63
100	$6.88 \cdot 10^6$	$1.80 \cdot 10^7$	31.63	68.37
101	$3.36 \cdot 10^7$	$4.24 \cdot 10^7$	45.23	54.77
102	$4.10 \cdot 10^7$	$3.05 \cdot 10^7$	57.29	42.71

5.8 Sample Complexity and Deconvolution

Supplementary Figure 18 shows sections of deconvoluted chromatograms with increasing sample complexity. A) 2 analytes, B) 3 analytes, C) 4 analytes, and D) 5 analytes. As can be seen from these chromatograms even with increasing sample complexity the deviation does not increase. It has to be pointed out that with increasing sample complexity more repetitive injections are necessary as described by equations 2 and 3.

Supplementary Figure 18. The figures show sections of deconvoluted chromatograms with increasing sample complexity. A) 2 analytes, B) 3 analytes, C) 4 analytes, and D) 5 analytes.

6 References

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